heavily pigmented cells along the free pupillary margin of the dorsal iris increase in size, become depigmented, undergo mitosis and form a vesicle that develops into a lens (Wachs, 1914, and others). When the latter becomes detached the cells in the dorsal iris regain their normal appearance. If varying amounts of dorsal iris are replaced by non-lens regenerating ventral iris, two widely separated lenses develop from the remaining dorsal pupillary margins after removal of the original lens. Secondary pupils experimentally produced in various regions of the dorsal iris by the insertion of pieces of pliofilm or cornea show that potentiality for lens formation is quite widely distributed and not confined to the free pupillary margin (Stone and Vultee, 1949).

These changes in the pigment cells can be experimentally inhibited by the following: 1) the presence of a transplanted normal lens of the same or another species (Stone, 1945); 2) the presence of a thirty-day lens regenerate (Stone, 1943); 3) injections of aqueous humor from eyes containing lenses (Stone and Vultee, 1949); 4) the presence of some carcinogens.

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X-Ray Effects on Mouse Pigmentation as Related to Melanoblast Distribution.

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Several features of the x-ray-induced greying response suggest certain properties of the pigmentation system. Follicles react largely as independent units, producing fully pigmented hairs (no apparent gross effect), white hairs, or infrequently mosaics. The effect is permanent or toward more white hairs in successive hair generations of the particular follicle (Chase, Quastler, and Skaggs, 1947; Chase, 1949). The percentage of follicles producing only white hairs after treatment increases with dose (200-1000 r) and with dose-rate. The percentage for a given dose is greater for follicles treated in catagen and telogen stages and for follicles of the smaller hair types (Chase, 1949; Chase and Rauch, in press). In a mosaic some of the medullary and cortical cells lack pigment granules entirely, some have a full complement, and some have a reduced number of granules. These various cells are arranged irregularly throughout the length of the hair. With increasing size of hairs (such as awls in the mouse, most of the hairs in rabbits and cats, all of the hairs in guinea pigs), the follicles are less "sensitive" to a given dose of x-radiation, produce a greater frequency of mosaics, and display less difference in response when treated in telogen or in anagen phases. It would seem that susceptible elements must be few in number, very few in smaller follicles. At beginning of anagen there would be a moderate increase of these elements but little, if any, further increase during the anagen phase (2 to 17 days post-plucking). After the initial supply for the original follicle invagination, there would be no new invasion for subsequent hair generations.

Following Masson's (1948) definitions the dendritic cells which eventually produce pigment are termed melanoblasts whereas the recipient cells, if any, are termed melanophores. Melanoblasts generally are not observed because of their fragility with certain standard histological methods and because they are largely obscured after the matrix cells become pigmented. Phase microscopy with the Spencer B minus contrast low, oil immersion objective on unstained frozen sections has proved most revealing. In young mice, melanoblasts of the basal layer of the skin epidermis can be seen to be incorporated in the original invaginations of newly-forming follicles. In early anagen stages of subsequent hair generations, melanoblasts are found in the permanent external sheath (continuous with basal layer of skin epidermis) or in the derivative basal layer of the bulb. They become melanogenic with fine dispersed granules and send long dendritic processes to the matrix cells. Later, beginning about 6 days post-plucking, the "inoc-

ulated" matrix cells (= melanophores) exhibit characteristic dense pigmentation. The keratinized hair shaft arises from these matrix cells. Pigmented matrix cells seldom divide, but new cells arise from the proliferating lower bulb and receive pigment as they pass by the melanoblast processes of the upper bulb. As few as 4 melanoblasts have been observed to supply the stream of matrix cells of a small follicle (zigzag) and the presence of only one or two results in a mosaic.

From histological and x-radiation evidence it appears that the "reservoir" of melanoblasts is within the epidermal follicle (cf. Taylor, 1949). Dermal melanophores apparently do not contribute to pigmentation in the hair follicle. The epidermal melanoblasts are the original granule-forming cells, but in the recipient matrix cells, the characteristic shape and color of the phenotype are further imposed upon the granules. Whereas biotin deficiency, for instance, causes a failure of melanin-formation on these granule sites (Chase and Rauch, in press), x-radiation destroys the source of supply of granules, namely, the epidermal melanoblasts of the follicle.

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A Comparative Colorimetric Study of Dopa-melanin Formation by Melanomas and Pigmented Skins.

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The costo-vertebral pigmented skin spot of the Syrian hamster, which contains both intradermal dentritic pigmented cells and exceptionally large pigmented hair roots[1] has proved to be consistently dopa-negative in histological sections prepared according to several standard technical procedures[2,3]. A method utilizing photo-electric colorimetric evaluation of dopa-melanin has been evolved which permits comparison of this pigmented tissue with other tissues which can be shown by histological means to be dopa-positive.

Skin specimens were surgically removed, freed of hair and unpigmented skin, chilled to 4° C., minced in a few drops of phosphate buffer, and rapidly weighed on small squares of clean coverslip using a microtorsion balance. Increasing weights of minced tissues were placed in centrifuge tubes containing 2 ml. of freshly prepared 1:1000 1-dopa solution, buffered to pH 7.4, and thoroughly mixed. After four hours' incubation at 37° C. the tubes were centrifuged at 25,000 rpm. for 15 minutes. The supernatant fluid was decanted, diluted to a standard volume, and kept in chilled colorimeter tubes until read on a Klett-Summerson photo-electric colorimeter using a KS-42 filter. The auto-oxidative rate of dopa was determined for each experiment by means of a control tube containing only buffered dopa. The control tube was given a value of 100% and the values obtained for the different tissue samples were compared with it.

Using this technique, experiments were done on tissues from the Cloudman mouse melanoma and from human melanomas. These tumor tissues gave almost straight line curves with the activity (color formation) increasing directly with the weight of the tissue samples. The results agree favorably with those obtained from Warburg studies of the enzyme activity of such melanoma tissues[4].

A number of types of skin were studied by this method. These included skin from the neck, flank and pigment spot of the hamster, pigmented nipples from guinea pigs, neck and flank skin from mice, adult and infant human skin, including depigmented and hyper-pigmented negro skin. In contrast to the straight line activity curve of the tumor tissue, these skins showed a biphasic activity curve with the final inclination in the downward direction. This characteristic of pigmented skins has been interpreted to indicate the presence of inhibitory substances which interfere with the formation of dopa-melanin. This observation is in agreement with previous work showing such inhibitory substances to be present in the skins of guinea pigs[5], rabbits[6,7] and man[8,9].

When the conditions of the experiment were changed so that the weight of the tissue was held constant and the concentration, but not the volume, of the dopa solution was increased, a curve was obtained which is suggestive of the classic "substrate competition" curve. The addition of a sulfhydril substance, glutathione[10] increased the inhibition of color formation in dilute dopa solutions but did not significantly affect higher concentrations.